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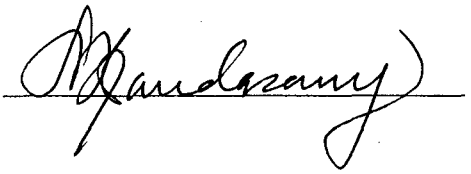

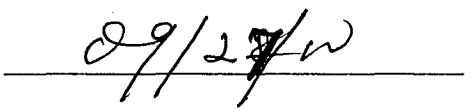

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13. ABSTRACT *(Maximum 200 Words)*

The goal of this project is to isolate the pie gene from Drosophila melanogaster and determine its function in cell death and cell survival. In the first year, we determined that pie mutations affected a 120kb region of Drosophila DNA. Now we have identified the specific transcription unit encoding pie function. The predicted PIE protein is a 600 amino acid polypeptide unrelated to other known proteins.

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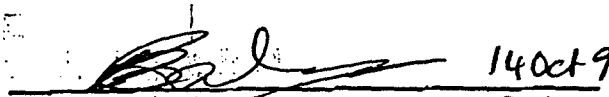

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Introduction

Changes in cell proliferation and cell survival are thought to be major fundamental causes of cancers. Cell proliferation and cell survival are normally important for growth and morphogenesis. Very little is known about how growth and morphogenesis are regulated in vivo, what genes are involved, and the relationship of these genes to carcinogenesis.

Although intracellular mechanisms of growth and survival have been studied extensively in tissue culture cells, these cells do not normally show regulated growth and morphogenesis. Regulation of these processes must be studied in vivo. The fruitfly Drosophila melanogaster provides a multicellular model organism suitable for genetic identification of growth and morphogenesis through study of mutations. We have isolated mutations that affect cell number in the developing Drosophila eye. These identify a candidate gene for growth regulation in vivo.

Each Drosophila compound eye comprises a precise array of about 800 ommatidia ("unit eyes"). Each ommatidium is constructed by 22 cells. Individual cell types are specified after a nearly random proliferative phase with no detectable role of lineage[1][2][3]. Each ommatidium arises through a sequence of post-mitotic single-cell inductions, each successive cell inducing the next[4][5][6]. How can the undifferentiated retinal epithelium contain a multiple of 22 cells, prior to their specification by induction? A clue came from studies of mutations in which too few ommatidia form[7]. In normal development, the first five cells of each ommatidium are specified during an arrest in G1 of the cell cycle. Remaining cells reenter the cell cycle, and are recruited to form the remaining ommatidial cells after another division. In mutants where very few ommatidia initiate differentiation, all the remaining cells reenter the cell cycle, but almost all then arrest in G2. Exceptions are cells in contact with the few differentiating ommatidia, which proceed through mitosis. This established the existence of an inductive signal from the first five cells of each ommatidium, permitting nearby cells to pass the G2/M checkpoint. The supernumerary, G2 arrested cells subsequently undergo cell death. We further showed that such cell death occurs even if supernumerary cells were arrested in the prior G1 phase, and so was not dependent on the particular cell cycle stage (our unpublished results). This indicated that the first five cells of each ommatidium also provide a survival signal for nearby cells. We therefore concluded that the first five cells of each ommatidium control cell number by "counting" the cells in their neighborhood, through local control of cell division and cell survival.

We performed mutations to identify mutations affecting Drosophila eye development[8]. A recessive mutation mapping at 43 centimorgans along the second chromosome has "rough" eyes and was named *pineapple eye* (*pie*). In *pie* mutants the retina has too few cells, because of excess cell death during eye development. Therefore the *pie* gene is a candidate to encode a component of the hypothesized survival signal. We proposed to clone the *pie* gene and characterize the role of its product. In the last annual report, I described genetic and molecular characterization of the *pie* gene region, culminating in the definition of a ~120 kb interval within which the *pie* gene must reside, the mapping of an inversion breakpoint that was shown to disrupt *pie* gene function, and the finding that *pie* is allelic to the *l(2)31Ek* locus.

Experimental methods

Northern analysis, cDNA library screens, and genomic DNA sequence analysis were used to identify candidate genes within the 120 kb critical region, beginning in the vicinity of the inversion breakpoint known to disrupt *pie* gene function. Once candidate genes were identified, two methods were available to identify the correct gene. These were sequencing genomic DNA from chemically-induced *pie* mutants and comparison with the sequence of the respective progenitor chromosomes, and rescue of *pie* mutant phenotypes by germline DNA transformation. In fact the former has proved sufficient.

Results

Diverse Northern blotting and cDNA analyses identified three transcription units close to the inversion breakpoint present in the *l(2)31Ek*$^{G2\text{-}4}$ allele, but not in its progenitor chromosome (Figure 1). One is homologous to human Replication Factor C. One is a new cytokinesin-like gene. The third is a novel open reading frame, apparently unrelated to any sequences from any organism yet deposited in databases. Genomic DNA corresponding to two chemically induced *pie* mutations, *pie*^{EB3} and *pie*$^{E1\text{-}16}$, was PCR amplified, and sequenced, along with control DNA from the respective progenitor strains. For each mutation a single base substitution was identified compared to the control sequences, in both cases within the coding region for the novel third gene. In *pie*^{EB3}, residue 393 is replaced by a stop codon. In *pie*$^{E1\text{-}16}$, a frameshift in codon 205 predicts premature truncation after amino acid 220. Both substitutions were consistent with ethane-methyl sulfonate mutagenesis, and each predicted truncation of this open reading frame. These findings identify this novel gene as the *pie* gene (Figure 1).

No changes were identified in either the RFC gene or the cytokinesin-like gene, excluding roles for these loci in the *pie* phenotype. In addition, a stop codon within the RFC coding region has been found in all genomic and cDNA sequences examined, indicating that this is a transcribed pseudogene.

The *pie* gene is predicted (from cDNA analysis) to encode a 600 amino-acid protein without recognizable structural motifs. The sequence lacks predicted transmembrane, nuclear or mitochondrial import sequences and might encode a cytoplasmic protein. The predicted sequence is notably acidic and cysteine rich. Motif-search programs identify potential RING finger domains and BIR fingers, both features found in Inhibitor of Apoptosis proteins (IAPs)[9]. However, we are uncertain whether these predictions are valid or a spurious consequence of the many cysteines.

Discussion

Our original goals for the second 12 months of the project, as outlined in the original statement of work, were: Task 5, transcript analysis; Task 6, sequence determination; Task 7, proof of *pie* gene identity by mutant sequencing and/or transformation rescue. All of these goals have been achieved.

Our cDNA, northern, and sequence analysis identified three candidate genes close to the *l(2)31Ek*$^{G2\text{-}4}$ breakpoint, and mutant sequencing identified one of these genes as the *pie* locus. *pie* encodes a novel protein unrelated to other sequences yet

described from other organisms.

Within the next twelve months we will proceed with the preparation of *pie*-specific antisera, and the cellular and subcellular analysis of pie protein expression in wildtype and in mutant animals.

Key Research Accomplishments

The *pie* gene has been identified. The gene encodes a novel protein.

Reportable outcomes

Funding has been applied for based in part on this work. Applications are under review by DAMD (BCRP-99, IDEA), and by NIH (1 RO1 GM61230-01).

Conclusions

Identification of the *pie* gene this year will allow future exploration of its molecular role in cell death and survival.

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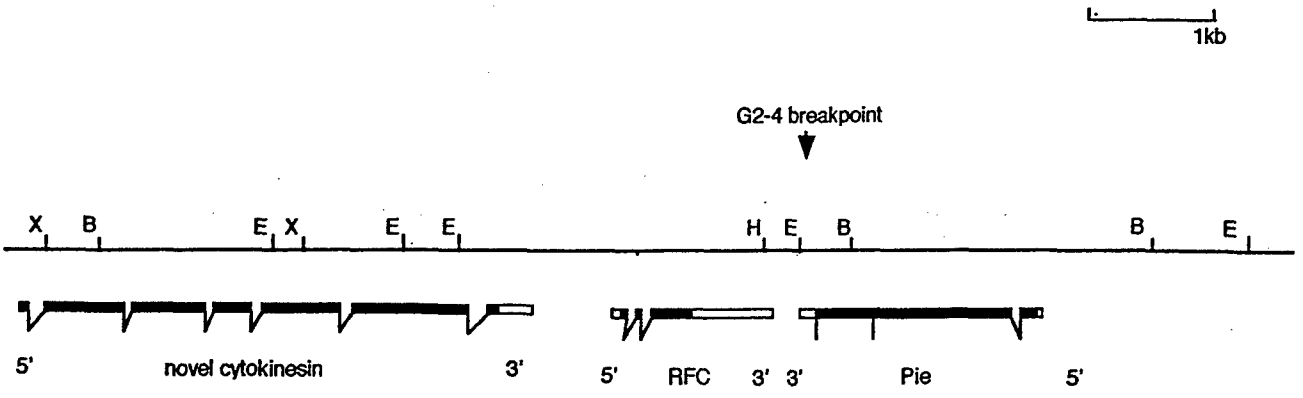

Figure 1 Transcriptional map of the *pie* gene region

Key

B - BamHI E - EcoRI H - HinDIII X - XbaI

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